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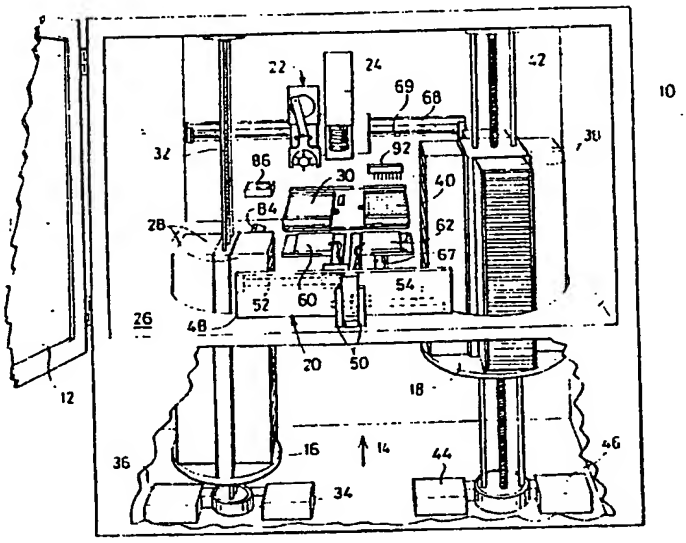
(54) Title: TRANSFER OF BIOLOGICAL SAMPLES

(57) Abstract

Apparatus for transferring biological samples from a random distribution in a petri plate (30) to the individual wells of a microtitre plate (40) comprises a vertically movable picker head assembly (22) including six equiangularly spaced needles (72) which are successively brought into a downwardly projecting operative position. The petri plate (30) and microtitre plate (40) are mounted on a holder (64) capable of horizontal sliding movement, orthogonal to the direction of sliding movement of the picker head assembly (22). An operative needle (72) of the picker head assembly (22) is positioned over a located biological sample in the petri plate (30), the picker head is moved downwardly so that the operative needle picks up the located biological sample, the picker head is raised, the picker head and the platform are shifted as necessary to position the picker head over a pre-selected well of the microtitre plate and the head is moved downwardly to deposit the biological sample in the well of the microtitre plate (40).



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Title: Transfer of Biological Samples

Field of the Invention

This invention relates to the transfer of biological samples, for example the transfer of cell colonies from a petri plate to the individual wells of a microtitre plate.

Background to the Invention

The invention was devised to provide an automatic colony picker apparatus and method for use in scientific research.

The process that must be carried out is the transference of cells from each of the colonies on a 90mm diameter petri plate (or dish) to the individual wells on a microtitre plate. The colonies can be as small as 0.5mm, as large as 3mm maximum dimension and grow in a random way with 50-1000 colonies per petri dish. The wells on the microtitre plate are organised in a 12 x 8 grid, with the centres 9mm apart. The procedure of transferring the colonies from the petri dish to the microtitre plate is currently done manually. Extreme concentration is required when picking the colonies as they are small and can be difficult to see.

The microtitre plates are first unpackaged from the sterile plastic wrapping. This can be done in a non-sterile environment so long as the lids are left on the

plates. Each plate is labelled using an indelible marker, writing on both the base and the lid of each of the petri dishes and microtitre plates. A written record is also kept for each of the microtitre well plates, detailing from which petri dish the colonies in each well came. The plates are then transferred to a flow hood where the plates can be opened without fear of cross-contamination or external contamination. The microtitre wells are then filled with a growing medium using a filler that accurately dispenses an amount of liquid into each well.

Cells from each of the colonies in the petri dish must be transferred to the microtitre plate on a one colony per well basis. In addition cross-contamination (ie putting cells from more than one colony into a well) must be avoided. This is done by using sterile toothpicks and using a new toothpick for each colony. The operation to be repeated involves:

1) Pick up a new sterilised toothpick

2) Push the toothpick into a colony on the petri dish in order to pick up some cells.

3) Transfer to a well and twizzle the toothpick in one of the microtitre wells to transfer the cells to the well.

4) Throw away the toothpick

5) Repeat from step 1

This task is mentally fatiguing and there is an upper limit of around 1500 picks per day. The operation must be

carried out under a flow hood to ensure that the wells are not contaminated from the environment.

The known picking procedure introduces a considerable bottleneck into the DNA sequencing and mapping processes. It is difficult to recruit staff to carry out the tedious job, which leads to the situation where the scientists who require the picking to be done must do it themselves. The process aims to isolate a particular gene and is probablistic, ie the more picks that are done the greater the probability of isolating the required gene. A typical experiment may involve up to 50,000 picks so a considerable amount of time must be spent picking. Scientists are therefore faced with doing this picking or not doing the experiment at all. In other words the picking process acts as a considerable disincentive to carry out many experiments.

The lack of an adequate machine and the evident demand for a machine to carry out the picking process lie behind the development of the invention.

Disclosure of the Invention

According to one aspect of the invention apparatus for transferring biological samples from a first receptacle to a second receptacle comprises a head carrying a plurality of projecting needles, support means for supporting the receptacles, first means for moving the head between a plurality of positions in each of which a corresponding one of the needles is presented in an operative position, second means for effecting relative movement between the head and the support means to bring the head either into a collecting position, in which the head is placed in

operative relationship with the first receptacle, or a depositing position in which the head is placed in operative relationship with the second receptacle, and third means for:

a) effecting relative movement between the head, when in the collecting position, and the support means to cause the operative needle to enter the first receptacle and then to withdraw from the first receptacle, whereby the needle picks up a sample from the first receptacle,

b) effecting relative movement between the head, when in the depositing position, and the support means to cause the operative needle to enter the second receptacle and then to withdraw therefrom, whereby the needle deposits the sample in the second receptacle,

the first and third means being operated alternately in sequence to load the needles with respective samples, the second means then being operated and the first and third means then being operated alternately in sequence to deposit the loaded samples in the second receptacle.

Preferably, the first means effect rotary indexing movement of the head, and the needles project radially of the axis of rotation of the head. In this case, the head may be positioned above the support means with each needle when in the operative position projecting downwardly, with the third means effecting vertical reciprocating movement of the head.

In the preferred embodiment the second means provide for sliding movement of both the head and the support means in mutually perpendicular directions which may both be

orthogonal to the direction of reciprocation of the head.

A sterilising station is preferably provided for sterilising the needles before collection of the samples, the sterilising station conveniently including a bath of an organic compound such as ethanol, together with particles or beads to provide an abrasive cleaning action on the needles as the latter are spun in the sterilising bath. Alternatively, or in addition, the sterilising station may have an ultrasound generator to clean and sterilise the needles.

According to another aspect of the invention there is provided a method of transferring biological samples from a first receptacle to a second receptacle, comprising using a head having a plurality of projecting needles which are brought into use successively to pick up samples from the first receptacle, effecting relative movement between the head and the receptacles and then successively depositing individual samples from the respective needles into the second receptacle.

Apparatus according to the invention will now be described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 is a front perspective view of the apparatus,

Figure 2 is a plan view of the apparatus of Figure 1,

Figure 3 is a fragmentary perspective view of a portion of the apparatus of Figures 1 and 2,

Figure 4, consisting of Figures 4a to 4d, is a sequence of

views illustrating how the apparatus removes plates from a carousel,

Figure 5 illustrates how a plate is accurately located,

Figure 6 illustrates how each petri plate is identified, and

Figure 7 is a plan view illustrating how the apparatus divides the area of a petri plate into six frames to aid resolution by a viewing system.

Referring to Figure 1, the apparatus is surrounded by an outer casing 10 a portion of which has a closable hinged door 12. A sterile air flow passes upwardly through the casing 10 in the general direction of the arrow 14, and this flow is filtered to remove toxic constituents.

The apparatus includes two carousels 16, 18 between which is located plate handling equipment generally indicated at 20. The carousel 16 serves as an input carousel and accommodates petri plates and the carousel 18 serves as an output carousel for accommodating microtitre well plates. The plate handling equipment is capable of withdrawing a selected petri plate from the input carousel 16 and a selected microtitre plate from the output carousel, and shifting the selected petri plate and microtitre plate to a position beneath a picker head assembly 22 and a CCD camera 24. As will be described in detail hereinafter, the picker head assembly is capable of transferring colonies from the petri plate to the individual wells of the microtitre plate.

The casing 10 has an intermediate panel 26 formed with

large apertures to permit passage of the air flow and to accommodate the two spaced carousels 16, 18. The input carousel 16 accommodates two removable racks 28 angularly displaced by 180°, each having space to hold fifteen petri plates 30. The carousel 16 is mounted on a central lead screw 32 driven by a stepper motor 34 for accurate adjustment of the vertical position of the carousel 16 relative to the panel 26 and the plate handling equipment 20. A further motor 36 rotatably drives the carousel 16 to enable the chosen rack 28 to be brought in to register with the plate handling equipment 20.

Similarly, the output carousel 18 accommodates four removable racks 38 angularly displaced by 90°, each having space to hold fifteen microtitre plates 40. The carousel 18 is mounted on a further central lead screw 42 driven by a further stepper motor 44 for accurate adjustment of the vertical position of the output carousel relative to the panel 26. A further motor 46 rotatably drives the output carousel 18 to enable the chosen rack 38 to be brought into register with the plate handling equipment 20.

Referring particularly to Figures 1 and 2, the plate handling equipment 20 includes a transverse plate 48 mounted for vertical shifting movement in guides 50. The plate 48 carries two horizontal slides 52, 54 from which project arms 56, 58 carrying respective suction plates 60, 62. The equipment 20 also includes a plate holder 64 capable of shifting movement fore and aft on a horizontal slide 66, under the control of a linear electric motor one component of which is shown at 67. In its forward plate-accessing position (Figure 2), the holder 64 is adjacent the carousels 16, 18 for plate removal and replacement, and in its rearward colony-transfer position (Figure 3) ,

the holder 64 supports a selected petri plate and a selected microtitre plate beneath the picker head and camera, as illustrated in Figure 3.

The picker head assembly 22 is capable of horizontal adjusting movement on a horizontal fixed slide 68 which also supports the camera 24 for sliding movement. The picker assembly 22 and the camera 24 are movable together along the slide 68, under the control of a linear electric motor one component of which is shown at 69. The picker head assembly 22 comprises a picker head 70 carrying six equiangularly spaced needles 72, a stepper motor being provided to rotate the head 70 about a horizontal axis so as to bring each needle 72 successively into an operative position in which the needle projects vertically downwardly. A further motor 74 drives a disc crank 76 and connecting rod 78 which have the effect of reciprocating the picker head 70 vertically in a direction orthogonal to both the direction of sliding movement of the picker head 22 and camera 24 along the slide 68, and the direction of sliding movement of the holder 64 along the slide 66.

Figure 4 shows how the plate handling equipment removes a selected petri plate 30 from the input carousel 16. In Figure 4a, the suction plate 60 is shown in its withdrawn position. The arm 56 is then driven along the slide 52 to insert the suction plate 60 into the selected recess in the input carousel. The application of suction to the plate 60 then causes the suction plate 60 to grip the lid of the selected petri plate 30 (Figure 4b) so that withdrawal of the arm along the slide 52 causes the selected petri plate to be removed from the carousel (Figure 4c) and dragged onto the holder 64 which is in its foremost position. Upward movement of the plate 48 in the

guides 50 then causes the lid of the petri plate to be removed (Figure 4d), ready for the latter to be shifted by movement of the holder 64 to the transfer station. It will be understood that the suction plate 62 operates in a corresponding manner to remove and replace selected microtitre plates from the output carousel.

Accurate location of a selected petri plate (or microtitre plate), is achieved by providing the holder 64 with location means in the form of a pivoted bar 80 (Figure 5) which, on accurate location of the plate, pivots against a spring so as to close a microswitch, a second microswitch being provided on the other side of the plate-receiving recess in the holder 64. The microswitches detect the presence or absence of the plate for error recovery purposes. A stop 82 on the holder 64 limits plate insertion This arrangement ensures that each loaded plate is positioned in the same place in the plate holder 64.

The locations of colonies in the petri plate are identified by the camera 24. To increase resolution, the area of the petri plate is sub-divided into six frames, marked 1 to 6 in Figure 7, the camera viewing the frames separately and sequentially. This is done by controlled shifting of the holder 64 along the guide 66 and shifting of the camera 24 along the guide 68. The images of the frame are processed by a controlling computer which computes the coordinates of the colonies. This information is used to drive the holder 64 (along the guide 66) and the picker head assembly (along the guide 68) until the picker head assembly is accurately located over the colony, ready for the latter to be picked by the operative needle of the picker head.

The petri plates are illuminated by means of a light source 84 (Figures 1 and 2) which is positioned beneath the holder and horizontally offset therefrom. The holder 64 has a rectangular cut-out to allow light to reach the petri plate 30. As a result, the illuminating light is directed obliquely upwards towards the petri plate 30. Light striking the colonies is reflected thereby, so that the camera 24 sees the colonies as light areas against a dark background. The oblique direction of the illuminating light improves contrast for certain types of plate, eg phage plates. For other types of plate, eg Ecoli, yeast, illumination is provided by a light source positioned above the holder. These two light sources are independently controlled.

A sterilising bath 86 (Figures 1 and 2) is provided to sterilise the picker needles 72. The bath contains 70% ethanol and glass beads 0.5mm diameter. After depositing the six colonies in the individual wells of the microtitre plate, the picker head is moved so that the needles dip into the sterilising bath 86. The head 70 is then rotated to clean the needles 72, the beads providing an abrading action. The picker head 70 is then ready to pick up six more colonies from the petri plate.

Figure 6 shows how an identification code 88 on the side of the petri plate is viewed by the camera with the aid of a mirror 90, so that the identification of the selected petri plate can be fed into the controlling computer software. A similar arrangement can be used to enter the identity of the microtitre plate into the software.

The apparatus functions in the following manner. The input carousel 16 is loaded with petri plates, each having

colonies suspended in agar gel. These colonies can be as small as 0.5mm. By use of the apparatus, the colonies are transferred from the random order of the agar gel into a defined grid in the wells of the microtitre plates so that the colonies can grow and further analysing may be carried out. The microtitre plates (at this stage empty) are loaded into the output carousel 18.

The rotational drive and axial shifting movement that can be applied to each carousel means that a selected petri plate and a selected microtitre plate can be brought into register with the plate handling equipment 20, and the selected plate removed from the carousel as necessary.

The selected petri plate is removed from the input carousel 16, as described with reference to Figure 4, and the selected microtitre plate is similarly removed from the output carousel 18. With the lids of both petri plate and microtitre plate removed (as in Figure 4d), the holder 64 is shifted rearwardly to carry the selected petri plate and microtitre plate to the picking station, as illustrated in Figure 3. At this stage, the individual wells of the microtitre plate are filled with appropriate medium, by means of a filler 92 shown Figures 1 and 2. The filler has an eight-way manifold and delivers liquid medium to eight microtitre wells at a time. The amount of liquid delivered is controlled by a pinch valve which controls the flow of the pressurised liquid. Sensors indicate when either the pressure drops or the liquid level is too low.

The light source 84 is energised to illuminate the petri plate with the obliquely directed light, and the six divisions or frames of the petri plate are viewed

successively by the camera 24, as a result of controlled shifting of the holder 64 and camera 24, as described with reference to Figure 7. The locations of the colonies in the agar medium are thus determined. These locations are typically in the form of xy coordinates and this information is used by the controlling computer to move the picker head assembly 22 along the slide 68 and the holder 64 along the slide 66 to position the picker head assembly in a collecting position, with the downwardly projecting needle 72 immediately over the location of the first colony to be picked. Energisation of the stepper motor then causes the picker head 70 to undergo a vertical reciprocating movement. This brings the needle tip down into contact with the located colony which adheres to the needle and is then withdrawn from the agar on upward movement of the needle. The stepper motor is energised to rotate the head 70 through 60° to bring the next needle into an operative downwardly facing position. Movement of the picker head assembly 22 and holder 64 as necessary brings the new needle 72 into position above the next colony to be picked. Energisation of the motor then causes the new needle to pick up the located colony, and this process continues until all six needles are loaded with respective colonies.

The colonies are then deposited in the individual wells of the microtitre plate 40. This is done by shifting (as necessary) the picker head assembly 22 and holder 64 to bring the picker head into a depositing position, with one of the loaded needles above the first well of the microtitre plate. Energisation of the motor lowers the loaded needle into the microtitre well so as to deposit the colony into the medium in the well, and the needle is then raised. Rotation of the head 70 to present a fresh

loaded needle, and shifting of the picker head assembly 22 and holder 64 as necessary, are then carried out to deposit the second colony, this process continuing until all six colonies have been deposited in the respective six wells of the microtitre plate.

The picker head assembly is then shifted to the sterilising bath 86. Rotation of the head, whilst the needles 72 dip into the ethanol solution in the bath, clean the needles 72 ready for picking of a fresh set of six colonies from the petri plate 30.

When all colonies have been removed from the petri plate (or the micritotre plate wells are fully occupied by colonies) the holder 64 shifts to the forward position, the lid is replaced on the petri plate (or microtitre plate), the spent petri plate (or the fully charged microtitre plate) is replaced in the corresponding carousel, and a fresh petri plate (or microtitre plate) is removed from the carousel.

The controlling computer governs all movements of the parts and the application of suction to the suction plates 60, 62. The imaging system, of which the camera 24 forms part, is capable of distinguishing colonies from background and is able to discriminate between three different colony types, namely yeast colonies which are white, bacterial colonies which are either blue or white or phage plaques which are either blue or clear. Only one type of colouring will be present in any one petri plate.

The described apparatus is capable of picking and transferring colonies reliably, automatically and quickly.

In a modification, the head carries a plurality of vertical needles which are horizontally spaced and each of which has its own solenoid which can be energised to move the particular needle to its extended position. Instead of relying on suction, the plate handling means may employ a simple mechanical hook to engage behind the plates in order to move the latter. It is also possible to have a single carousel, accommodating both petri plates and microtitre plates, instead of the described pair of carousels.

Claims

1. Apparatus for transferring biological samples from a first receptacle to a second receptacle, comprising a head carrying a plurality of projecting needles, support means for supporting the receptacles, first means for moving the head between a plurality of positions in each of which a corresponding one of the needles is presented in an operative position, second means for effecting relative movement between the head and support means to bring the head either into a collecting position, in which the head is placed in operative relationship with the first receptacle, or a depositing position in which the head is placed in operative relationship with the second receptacle, and third means for

a) effecting relative movement between the head, when in the collecting position, and the support means to cause the operative needle to enter the first receptacle and then to withdraw from the first receptacle, whereby the needle picks up a sample from the first receptacle

b) effecting relative movement between the head, when in the depositing position, and the support means to cause the operative needle to enter the receptacle and then to withdraw therefrom, whereby the needle deposits the sample in the second receptacle,

the first and third means being operated alternately in

sequence to load the needles with respective samples, the second means then being operated and the first and third means then being operated alternately in sequence to deposit the loaded samples in the second receptacle.

2. Apparatus according to claim 1, wherein the first means effect rotary indexing movement of the head, and the needles project radially of the axis of rotation of the head.

3. Apparatus according to claim 2, wherein the head is positioned above the support means and each needle, when in the operative position, projects downwardly.

4. Apparatus according to claim 3, wherein the second means provide for sliding movement of both the head and the support means in mutually perpendicular directions.

5. Apparatus according to claim 3 or 4, wherein the third means provide for vertical reciprocating movement of the head.

6. Apparatus according to claim 4 or 5, wherein the head slides in a direction orthogonal to both the direction of sliding of the support means and the direction of reciprocation of the head.

7. Apparatus according to any of the preceding claims and including a sterilising station for sterilising the needles before collection of the samples.

8. Apparatus according to claim 7, wherein the sterilising station includes a bath of organic compound together with particles or beads to provide an abrasive

cleaning action on the needles.

9. Apparatus according to claim 8, wherein the support means comprise a plate holder shiftable to a plate accessing position at which plate handling means are located, and a carousel which is mounted for controlled rotation and shifting in a direction parallel to the axis of rotation to bring a selected receptacle stored in the carousel into register with the plate accessing position, at which receptacles are moved in and out of the carousel by the plate handling means.

10. Apparatus according to any of the preceding claims and including a camera for viewing the first receptacle, image processing means linked to the camera for deriving the coordinates of samples in the first receptacle, and illuminating means which direct light onto the first receptacle at an oblique angle to the viewing direction of the camera.

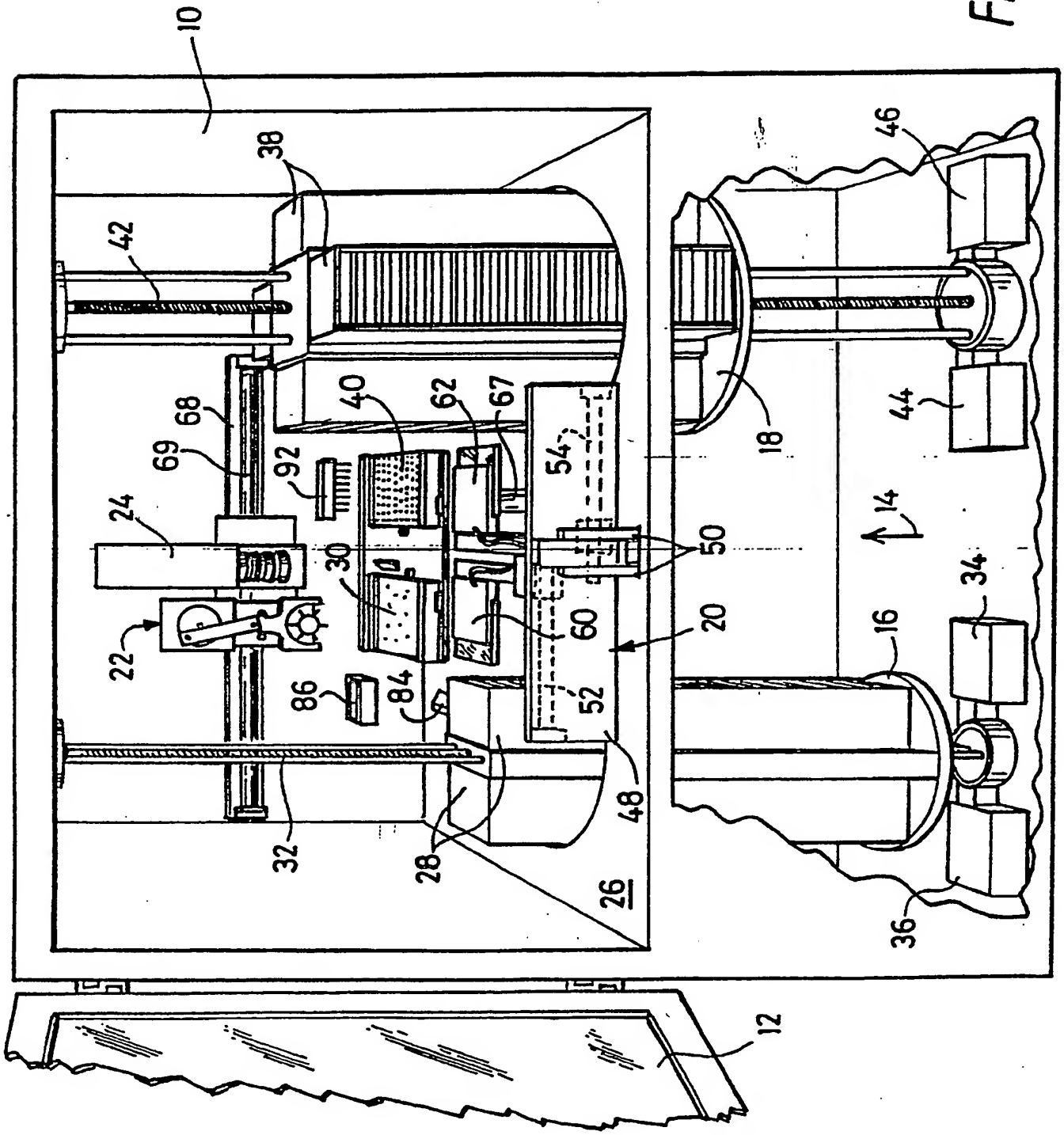
11. Apparatus according to any of the preceding claims and including means for delivering a sterile air flow past the components of the apparatus.

12. A method of transferring biological samples from a first receptacle to a second receptacle, comprises using a head having a plurality of projecting needles which are brought into use successively to pick up samples from the first receptacle, effecting relative movement between the head and the receptacles and then successively depositing individual samples from the respective needles into the second receptacle.

13. A method according to claim 12, wherein the first

receptacle is a petri plate and the second receptacle is a microtitre plate, the individual samples being deposited in the individual wells of the plate.

Fig. 1



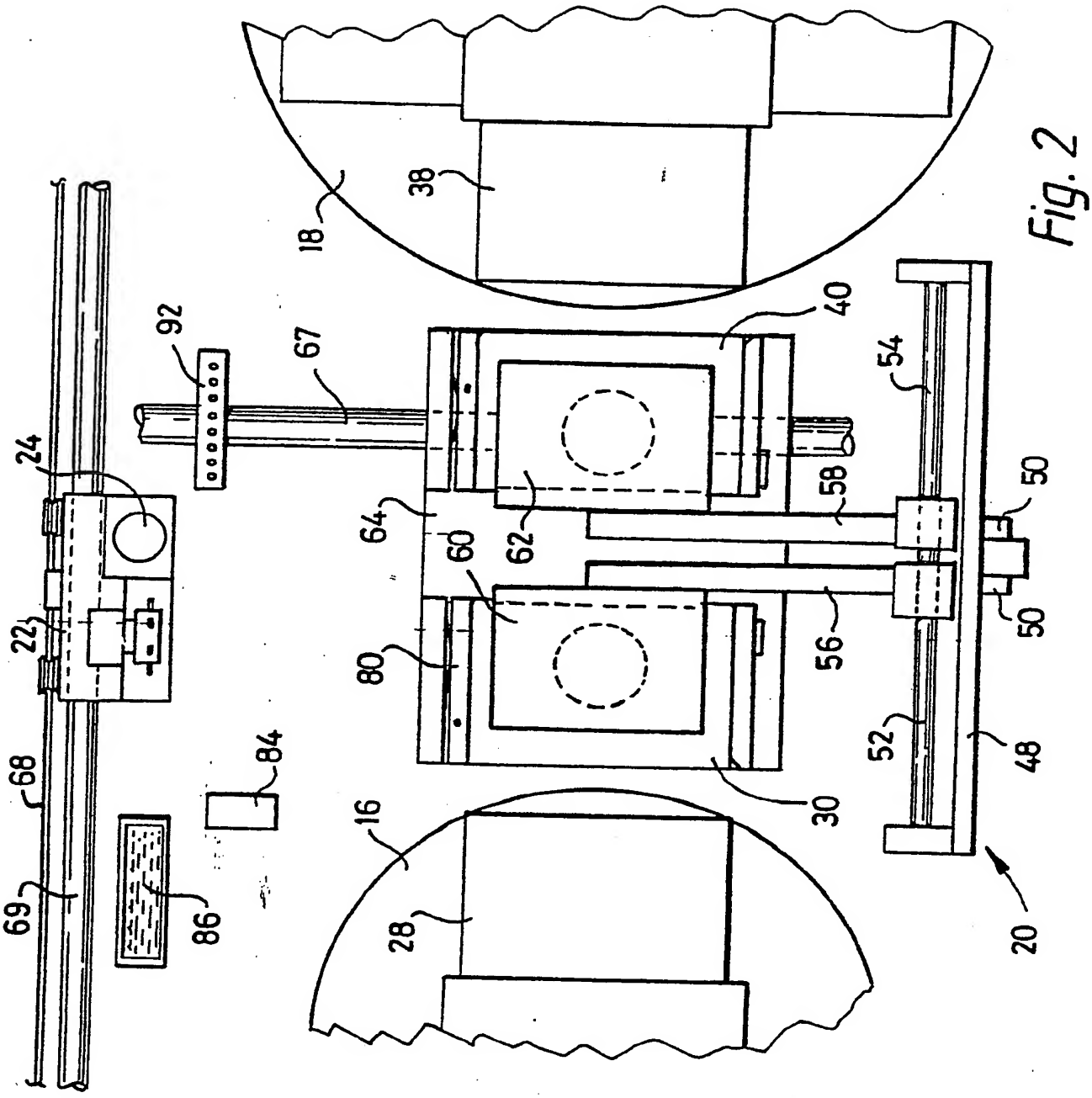

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Fig. 2

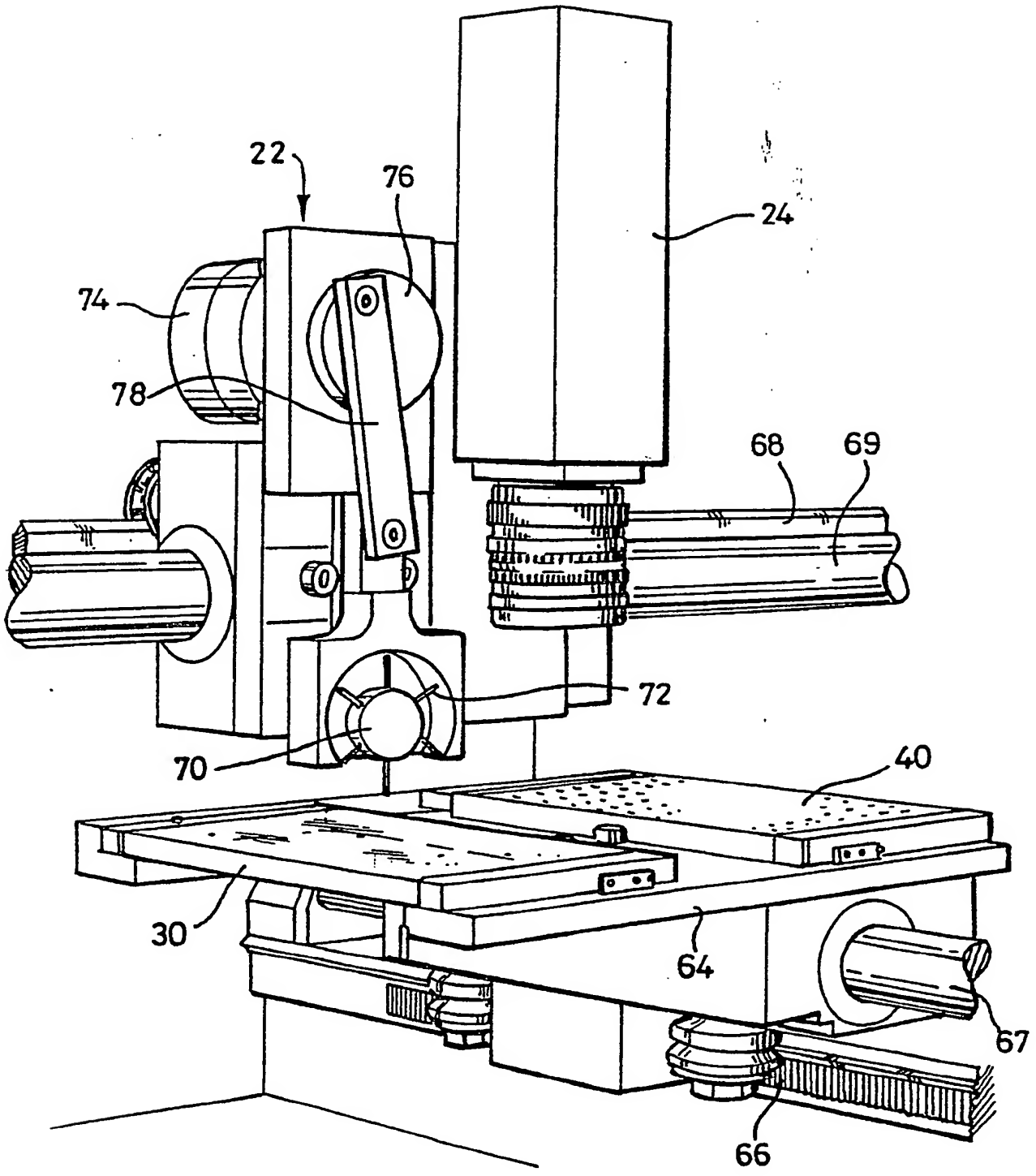

Fig. 3

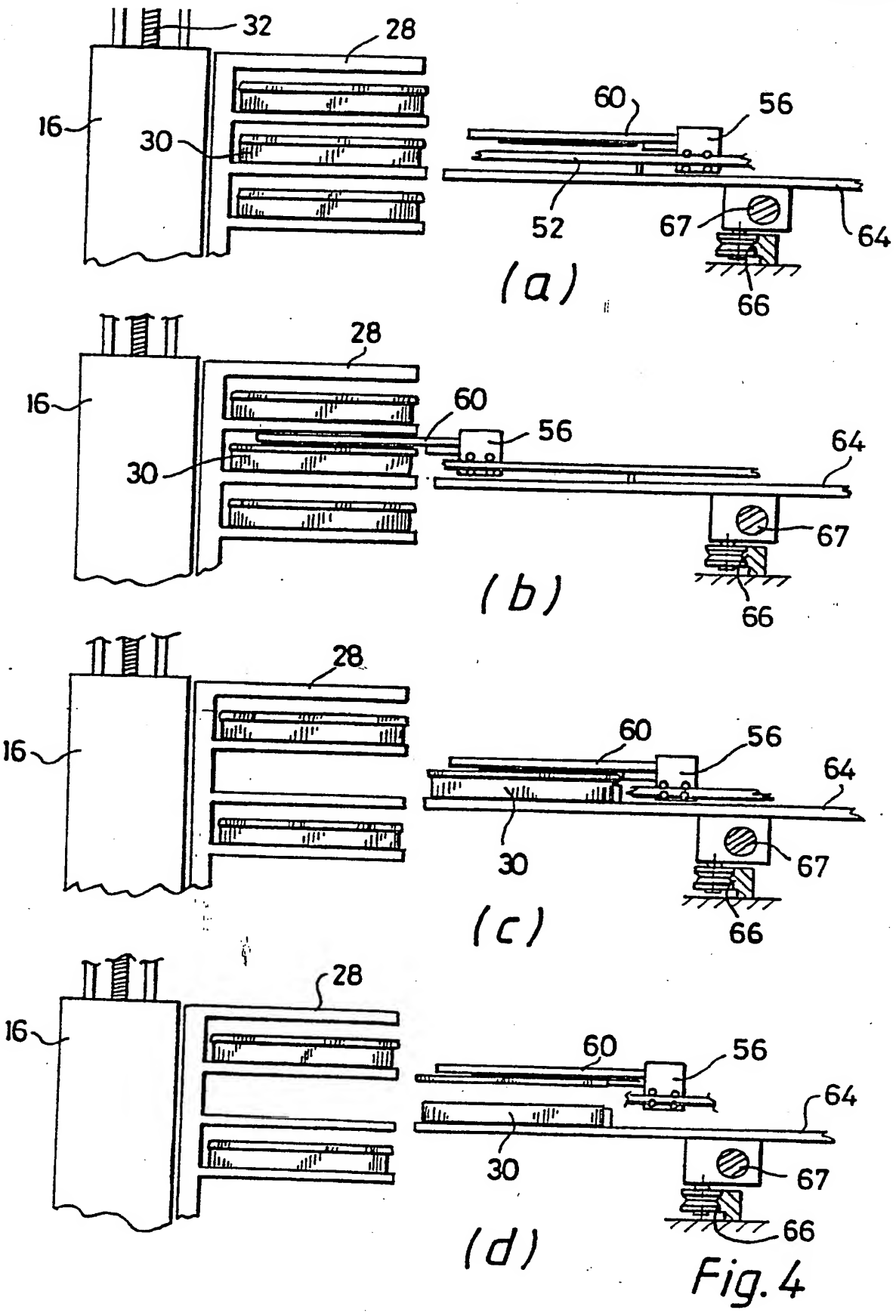
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Fig. 4

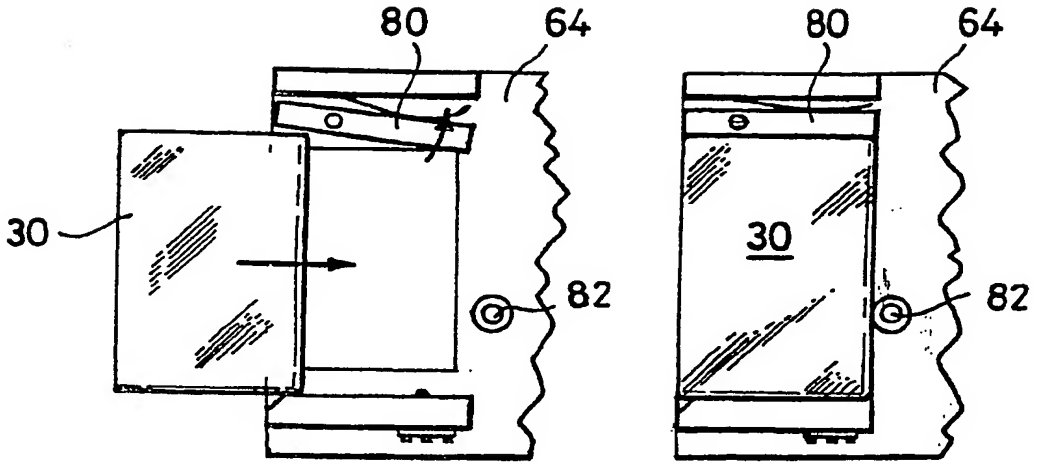

Fig. 5

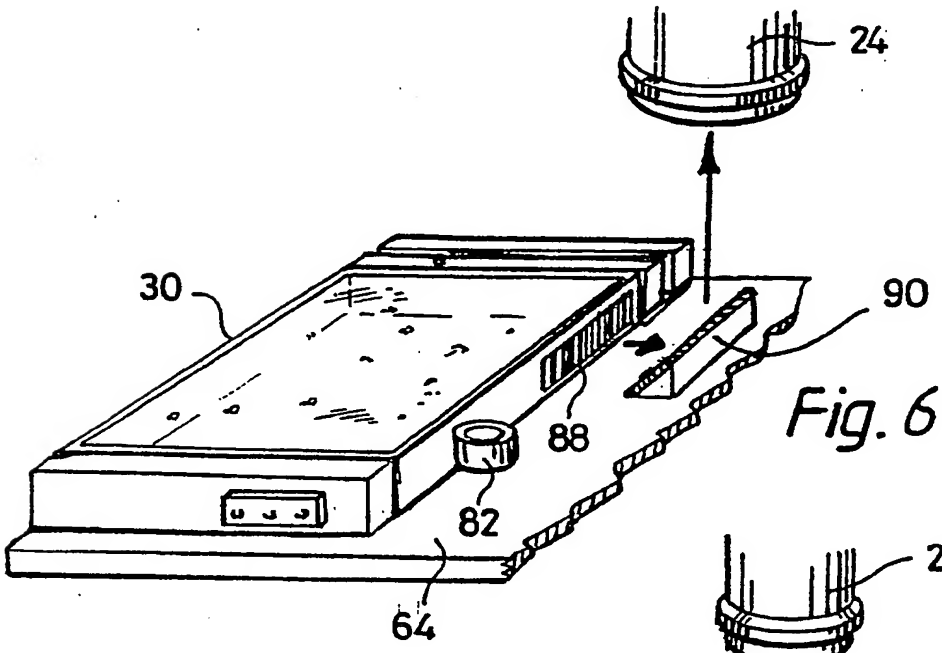

Fig. 6

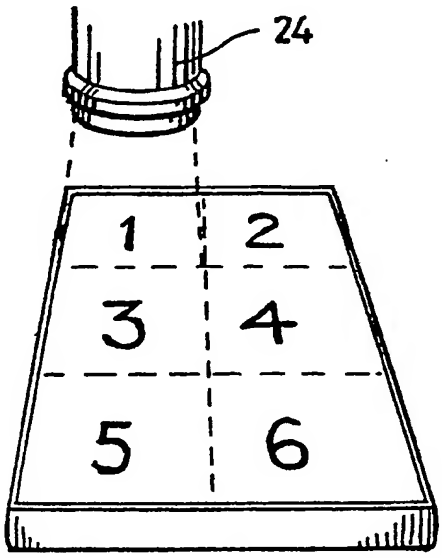

Fig. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/02319

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12M1/32; G01N35/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12M ; G01N ; B01L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 292 995 (SUMITO ELECTRIC INDUSTRIES LTD) 30 November 1988 see claims; figure 1	1
A	WO,A,8 705 323 (THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY) 11 September 1987 see claims; figures	1-3
A	FR,A,2 527 221 (HITACHI LTD ET AL.) 25 November 1983 see claims; figures	1

[10] Special categories of cited documents:

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 APRIL 1992	06 MAY 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	COUCKE A.O.M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9102319 SA 55104

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